

5 **WHAT IS CLAIMED:**

1. A method of rejuvenating a primary cell, comprising:
 - a. transferring a primary cell, the nucleus from said primary cell or chromosomes from a primary cell to a recipient oocyte or egg in order to generate an embryo;
 - 10 b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;
 - c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune-compromised animal to form a teratoma;
 - d. isolating said resulting teratoma;
 - 15 e. separating the different germ layers for the purpose of identifying specific cell types;
 - f. isolating a cell of the same type as the primary cell.
2. The method of Claim 1, wherein said primary cell is a senescent cell or a cell that is near senescence.
- 20 3. The method of Claim 1, wherein said cell isolated from said nuclear transfer teratoma has telomeres that are on average at least as long as those of cells from a same age control teratoma that is not generated by nuclear transfer techniques.
4. The method of Claim 4, wherein said telomeres are on average longer than those of cells from a same age control teratoma that is not generated by nuclear
- 25 transfer techniques.
5. The method of Claim 2, wherein said primary cell is a fibroblast.

5 6. The method of Claim 1, wherein said immune-compromised animal is
a SCU) or nude mouse.

7. The method of Claim 1, wherein said primary cell has at least one
alteration to the genome.

8. A method of making a primary cell having the same genotype as a first ✓
10 cell which is of a different cell type, comprising:

- a. transferring the nucleus from said first cell to a recipient oocyte in
order to generate an embryo;
- b. obtaining an inner cell mass, embryonic disc and/or stem cell using
said embryo;
- 15 c. injecting said inner cell mass, embryonic disc and/or stem cell into an
immune compromised animal to form a teratoma;
- d. isolating said resulting teratoma;
- e. separating the different germ layers for the purpose of identifying
specific cell types;
- 20 f. isolating a cell of a different type than the first cell, wherein the
telomeres of said new primary cell are at least as long the telomeres of
a same age control cell in a teratoma not generated by nuclear transfer
techniques.

9. The method of Claim 8, wherein said first cell is a senescent cell or a
25 cell that is near senescence.

10. The method of Claim 9, wherein said first cell is a fibroblast.

5 11. The method of Claim 8, wherein said primary cell is of a type selected from the group consisting of smooth muscle, skeletal muscle, cardiac muscle, skin and kidney.

 12. The method of Claim 8, further comprising growing said cell of a different type in the presence of growth factors to facilitate further differentiation.

10 13. The method of Claim 11, wherein said primary cell is used to generate a tissue (for transplantation into a patient in need of a transplant).

 14. The method of Claim 8, wherein the genome of the first cell is altered prior to nuclear transfer.

 15. The cell isolated by the method of Claim 8.

15 16. The tissue isolated by the method of Claim 13.

 17. The method of Claim 7, wherein said genetic alteration comprises the transfection of at least one heterologous gene.

 18. The method of Claim 7, wherein said genetic alteration comprises the disruption of at least one native gene.

20 19. The method of Claim 14, wherein said genetic alteration comprises the transfection of at least one heterologous gene.

 20. The method of Claim 14, wherein said genetic alteration comprises the disruption of at least one native gene.

25 21. A method of performing compound genetic manipulations in a primary cell, comprising rejuvenating said primary cell between genetic manipulations using nuclear transfer into a recipient oocyte, wherein said cell is passaged to a senescent or near-senescent state prior to nuclear transfer.

5 22. A method of performing compound genetic manipulations in a primary ✓
cell, comprising rejuvenating said primary cell between genetic manipulations using
nuclear transfer into a recipient oocyte, wherein said cell is induced into a senescent-
like or near-senescent-like state prior to nuclear transfer.

10 23. The method of Claim 21, whereby rejuvenation results in an
embryonic cell that has telomeres at least as long on average as a same age control
embryonic cell.

 24. A primary cell that has been genetically altered according to the
method of Claim 21.

15 25. A method of making a genetically altered animal having the same
genotype as the cell of Claim 24, comprising

- a. transferring the nucleus of said cell into a recipient oocyte,
- b. generating an embryo or embryonic stem cell from said nucleated
oocyte,
- c. introducing said embryo or embryonic stem cell into a recipient
20 female, and
- d. allowing said embryo or embryonic stem cell to fully develop such that
said female delivers a newborn animal having the same genotype as
said primary cell.

25 26. The genetically altered animal produced by the method of Claim 25,
whereby said animal has telomeres that are at least as long on average as a same age
control animal.

5 27. A method of re-cloning a cloned animal using nuclear transfer techniques, wherein the donor cell used to supply the nucleus of the re-clone is a cell that is senescent or near senescence.

 28. The method of Claim 25, wherein said re-cloned animal has been genetically altered with respect to the cloned animal.

10 29. A method of making a re-cloned inner cell mass, blastocyst, teratoma embryo, fetus or animal containing at least two genetic modifications, comprising:

- a. obtaining a primary cell from an animal of interest,
- b. making a first genetic modification to said primary cell by inserting heterologous DNA and/or deleting native DNA,
- 15 c. allowing said genetically modified primary cell to multiply to senescence or near-senescence,
- d. using a first genetically modified senescent or near-senescent cell as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg,
- 20 e. obtaining a cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first genetic modification,
- f. obtaining a cloned primary cell from said cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal,
- g. making a second genetic modification to said cloned primary cell by
- 25 10 inserting heterologous DNA and/or deleting native DNA,
- h. allowing said second cloned primary cell to multiply until senescence or near senescence,

- 5 i. using a senescent or near-senescent cloned primary cell having said
first and second genetic modifications as a nuclear donor for nuclear
transfer to an enucleated oocyte or an enucleated fertilized egg, and
j. obtaining a re-cloned inner cell mass, blastocyst, teratoma, embryo,
fetus or animal having said first and second genetic modifications.

10 30. The method of Claim 29 further comprising steps where said re-cloned
inner cell mass, blastocyst, teratoma, embryo, fetus or animal is again re-cloned, and
wherein a third genetic modification is made such that the farther re-clone has the
first, second and third genetic modifications.

15 31. The method of Claim 30, wherein said further re-clone is generated by
nuclear transfer techniques using a senescent or near-senescent donor cell.

 32. The method of Claim 29, wherein said re-clone has telomeres that are
at least as long on average as a same age control animal that was not generated using
nuclear transfer techniques.

20 33. The method of Claim 31, wherein said farther re-clone has telomeres
that are at least as long on average as a same age control animal that was not
generated using nuclear transfer techniques.

 34. The method of Claim 29, wherein the genetic modifications involve
genes that are responsible for immunological function.

25 35. The method of Claim 29, wherein said animal of interest is an
ungulate.

 36. The method of Claim 35, wherein said animal of interest is a bovine.

5 37. A method of re-setting the life-span of senescent, checkpoint arrested, ✓
or near-senescent cells, comprising transferring the nucleus of said cell into a
recipient oocyte.

 38. The method of Claim 37 wherein said recipient oocyte is of a different
species than said senescent or near-senescent cell.

10 39. The method of Claim 37 further comprising generating an embryo or
embryonic stem cell from said nucleated oocyte.

 40. A method of identifying at least one gene that either directly or ✓
indirectly enhances telomerase activity, comprising screening a cDNA or mRNA
library generated from an embryo or embryonic stem cell for members that enhance
15 telomerase activity in a senescent or near-senescent cell.

 41. The method of Claim 40 whereby enhancement in telomerase activity
is measured by measuring for enhanced expression of a telomerase reporter gene.

 42. The method of Claim 41 wherein said telomerase reporter gene is
construct comprising the hTERT gene fused to a reporter gene.

20 43. The method of Claim 42 wherein the construct comprises a gene
fusion.

 44. The method of Claim 42 wherein the construct comprises a protein
fusion.

 45. The method of Claim 40 whereby enhanced telomerase activity is
25 measured via the TRAPeze assay.

46. The method of Claim 40 whereby said cDNA or mRNA library is subjected to subtractive hybridization with a cDNA or mRNA library from a senescent cell prior to library screening.

47. A method of identifying at least one gene that either directly or indirectly suppresses telomerase activity, comprising, screening a cDNA or mRNA library generated from a senescent or near-senescent cell for members that suppress telomerase activity in an embryonic stem cell.

48. The method of Claim 47 whereby a decrease in telomerase activity is measured by measuring for decreased expression of a telomerase reporter gene.

49. The method of Claim 47 wherein said telomerase reporter gene is a construct comprising the hTERT gene fused to a reporter gene.

50. The method of Claim 49 wherein the construct comprises a gene fusion.

51. The method of Claim 49 wherein the construct comprises a protein fusion.

52. The method of Claim 47 whereby telomerase activity is decreased via a protein interaction, and a decrease in telomerase activity is measured via the TRAPeze assay.

53. The method of Claim 47 whereby said cDNA or mRNA library is subjected to subtractive hybridization with a cDNA or mRNA library from an embryonic stem cell prior to library screening.

54. A method of identifying a protein that enhances EPC- 1 and/or telomerase activity, comprising

- 5 a. collecting fractions from the cytoplasm of an oocyte,
- b. adding them to a cell-free system designed from a senescent or near-senescent cell, and
- c. measuring for changes in telomerase and/or EPC- 1 activity that result from exposure to specific oocyte cytoplasmic fractions.

10 55. A gene identified by the method of Claim 40.

 56. A gene identified by the method of Claim 47.

 57. A protein identified by the method of Claim 54.

 58. A method for screening for compounds that inhibit telomerase and/or

EPC-1 activity, comprising exposing an embryonic stem cell generated by nuclear

15 transfer techniques using a senescent or near-senescent donor cell to a compound to determine whether said compound inhibits telomerase and/or EPC-1 activity.

 59. A compound identified by the method of Claim 58.

 60. A pharmaceutical composition comprising the gene of Claim 55, or a portion or a transcription product thereof, for the purpose of enhancing telomerase

20 activity in a subject in need of such enhanced activity.

 61. A pharmaceutical composition comprising the gene product encoded by the gene of Claim 55 for the purpose of enhancing telomerase activity in a subject in need of such enhanced activity.

 62. A pharmaceutical composition comprising the gene of Claim 56, or a
25 portion or a transcription product thereof, for the purpose of suppressing telomerase activity in a subject in need of such suppressed activity.

5 63. A pharmaceutical composition comprising the gene product encoded
by the gene of Claim 56 for the purpose of suppressing telomerase activity in a
subject in need of such suppressed activity.

 64. A pharmaceutical composition comprising the protein of Claim 58 for
the purpose of enhancing telomerase activity in a subject in need of such enhanced
10 activity.

 65. A gene encoding the protein of Claim 58.

 66. A pharmaceutical composition comprising the gene of Claim 65 for the
purpose of enhancing telomerase activity in a subject in need of such enhanced
activity.

15 67. A pharmaceutical composition comprising the compound of Claim 59
for the purpose of inhibiting telomerase activity in a patient in need of such decreased
activity.

 68. A method for activating endogenous telomerase and/or EPC-1 for the
purpose of extending the life-span of a primary cell.

20 69. A cell with rejuvenated proliferation potential produced by expressing
a cell committed to a somatic cell life-span or DNA thereof to a germ or embryonic
cell or fractionated compounds thereof.

 70. The cell of Claim 69 wherein said cell has increased EPC-1 activity
and/or lengthened telomere relative to an age-matched somatic cell of the same type
25 and species.

5 71. The cell of Claim 69 which is selected from the group consisting of
human, bovine, equine, canine, feline, porcine, mouse, rat, goat, sheep, guinea pig,
bear, rabbit.

10 72. The cell of Claim 69 which is a human cell.

10 73. DNA with extended telomeres derived from a cell according to Claim
69.

 74. The DNA of Claim 73 which is derived from a human cell.

 75. A method for producing a cell with rejuvenated proliferation potential
by exposing a cell committed to a somatic cell lineage or DNA therefrom to an egg,
oocyte, embryonic cell or fractionated components isolated therefrom.

15 76. The method of Claim 75 wherein said somatic cell is senescent, near-
senescent, or checkpoint arrested.

 77. The method of Claim 75 wherein said somatic cell is a human cell.

20 78. The method of Claim 77 wherein said somatic cell is obtained from a
person with an aging associated condition or a condition associated with increased
cell turnover.

 79. The method of Claim 78 wherein said condition is selected from the
group consisting of AIDS, muscular dystrophy, a neurodegenerative disorder,
hypertension, immune deficiency, osteoarthritis, and diabetes.

25 80. A cloned non-human embryo, animal cell or non-human animal
produced by nuclear transfer, wherein the donor cell or nucleus is a senescent cell or
checkpoint arrested cell.

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86. The cell of Claim 85, wherein said EPC-1 gene is operably linked to a CMV, PGK, or other non-EPC-1 regulatory sequence.

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